

Fig. 1

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GAAA
-4

AGACGCGCAGGCCGGGCGCTCTCCCGACGGGGAGTAGCGCTGCAGCCGGACGCAGGGTGCAGTTA
10 20 30 40 50 60

M G S K G G F I L L W L -14
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70 80 90 100 110 120

L S I L A V L C H L G H S L Q C Y 4
CTG TCC ATC CTG GCT GTT CTC TGC CAC TTA GGT CAC AGC CTG CAG TGC TAT
130 140 150 160 170

ψ
N C I N P A G S C T T A M N C S H 21
AAC TGT ATC AAC CCA GCT GGT AGC TGC ACT ACG GCC ATG AAT TGT TCA CAT
180 190 200 210 220

N Q D A C I F V E A V P P K T Y Y 38
AAT CAG GAT GCC TGT ATC TTC GTT GAA GCC GTG CCA CCC AAA ACT TAC TAC
230 240 250 260 270

Q C W R F D E C N F D F I S R N L 55
CAG TGT TGG AGG TTC GAT GAA TGC AAT TTC GAT TTC ATT TCG AGA AAC CTA
280 290 300 310 320

ψ
A E K K L K Y N C C R K D L C N K 72
GCG GAG AAG AAG CTG AAG TAC AAC TGC TGC CGG AAG GAC CTG TGT AAC AAG
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↓
S D A T I S S G K T A L L V I L L 89
AGT GAT GCC ACG ATT TCA TCA GGG AAA ACC GCT CTG CTG GTG ATC CTG CTG
380 390 400 410 420

L V A T W H F C L * 98
CTG GTA GCA ACC TGG CAC TTT TGT CTC TAA
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Fig 2

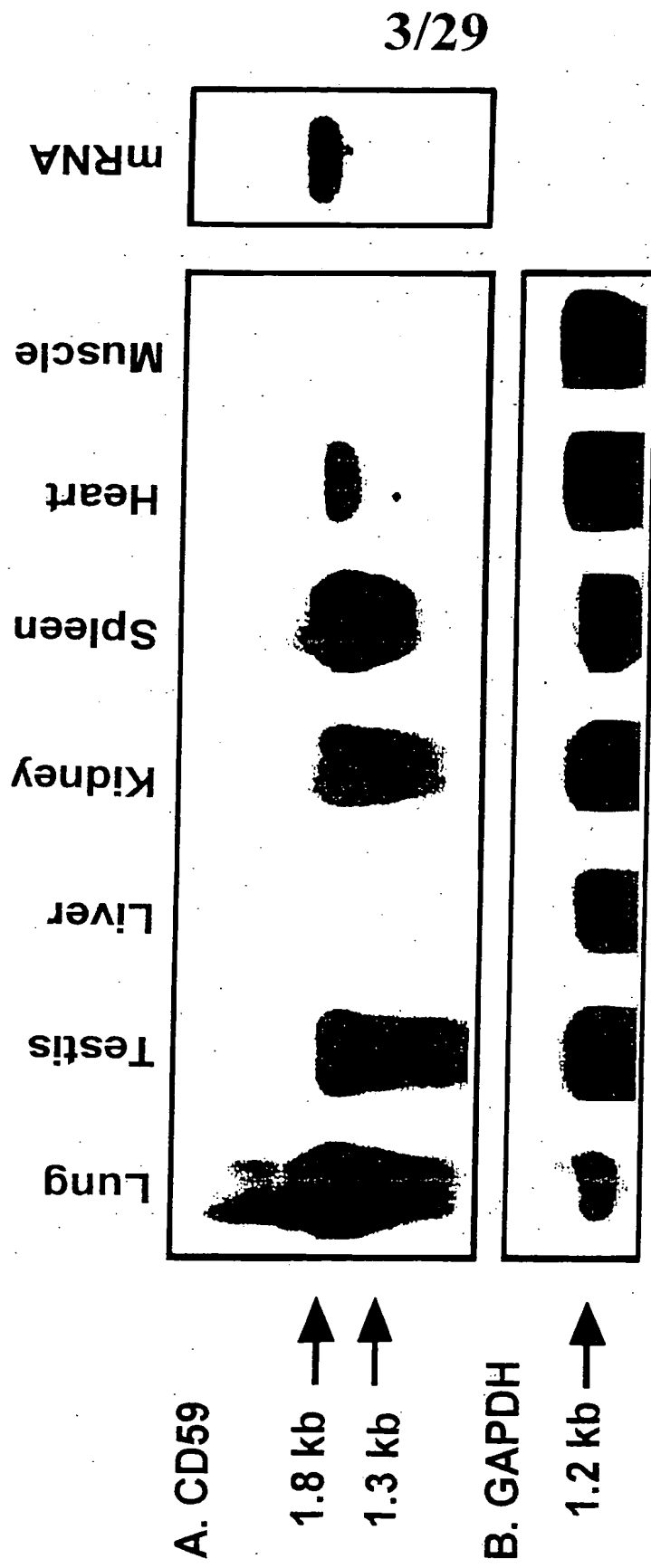


Fig. 3

	-20	-10	1	10	20	30
PIG:	MSGKGGFILLWLLSILAVLCHLGHSLQCYNCINP-AGSCTAMNCSHNQDACIFVEAVPPKTTYQ					
HUM:	MGIQGGSVLFGLLLVAVFCHSGHSLQCYNCNP-TADCKTAVNCSSDFDACLIITKAGLQVYN-K					
RAT:	MRARRGFIL--LLL-LAVLCSTGVSLRCYNCLDP-VSSCKTNSTCSPNLDACLAVAVSGKQVYQ-Q					
MUR:	MRAQRGLIL--LLLLAVFCSTAVSLTCYHCFQPVVSSCNMNSTCSPDQDSCLYAVAGMQVYQ-R					

	40	50	60	70	80	90
PIG:	CWRFDECNFD FISRNLA EKKLKYNCCKRDL CNKSD-----ATIS-SGKTALL-VILLVATWHFCL.					
HUM:	CWKFEHCNFNDVTTRLRENELTYYCCKKDL CNFNEQLEN--GGTSLSEKTVLLLVTPFLAAAWSLHP.					
RAT:	CWRFSDCNAKFILSRLEIANVQYRCCQADLCNKSFEDKPNNGAISLLGKTALL-VTSVLAAILKPCF.					
MUR:	CWKQSDCHGEIIMDQLEETKLKFRCCQFNLCNKSD-----GS-LGKTPLLGTSVLVAIL-NLCFLSHL.					
RAB:	CWRYEDCNFEFISNRLEENSLKYNCCKRDL CNGPEDDGTAL-----TGRTVLL-VAPLLAAARNLCL					

Fig. 4

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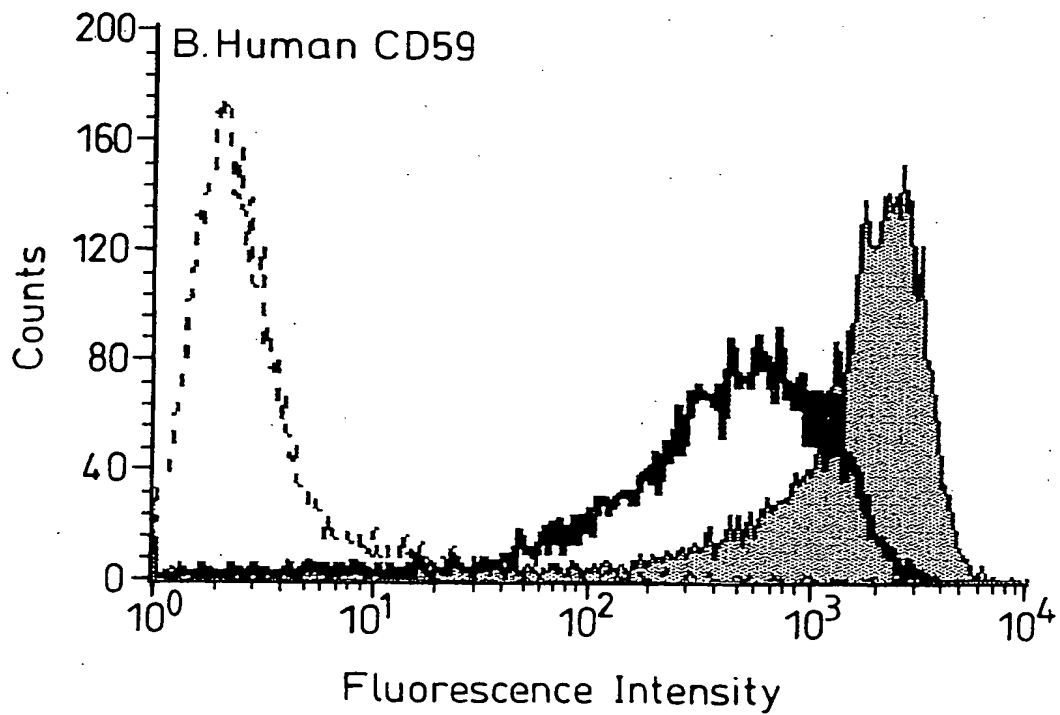
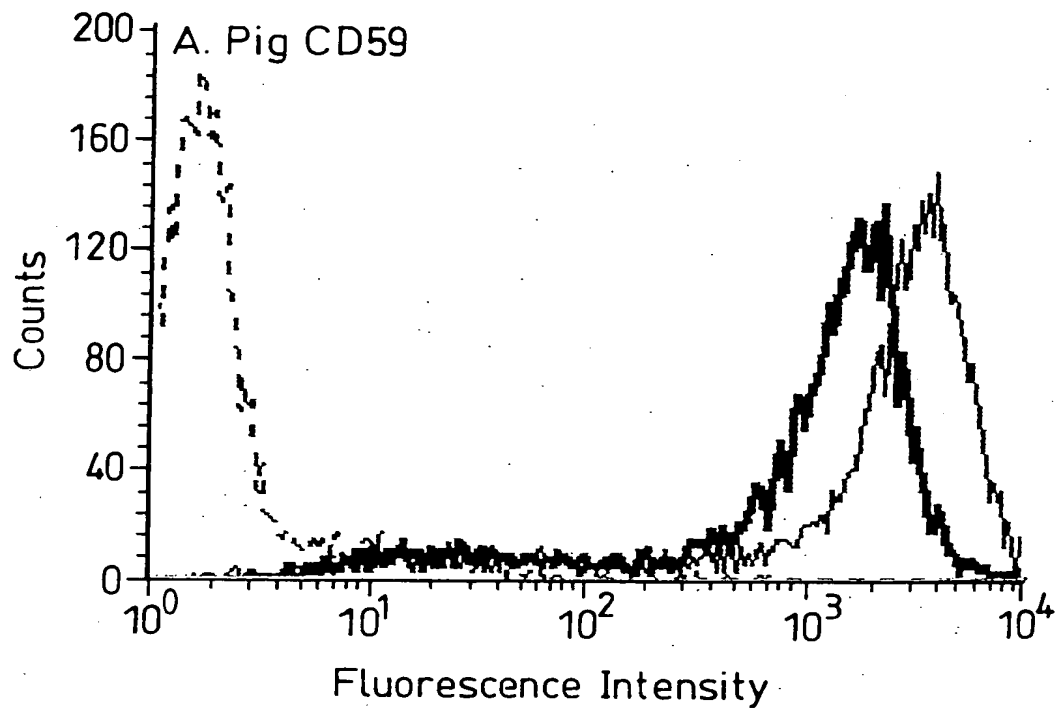


Fig 5

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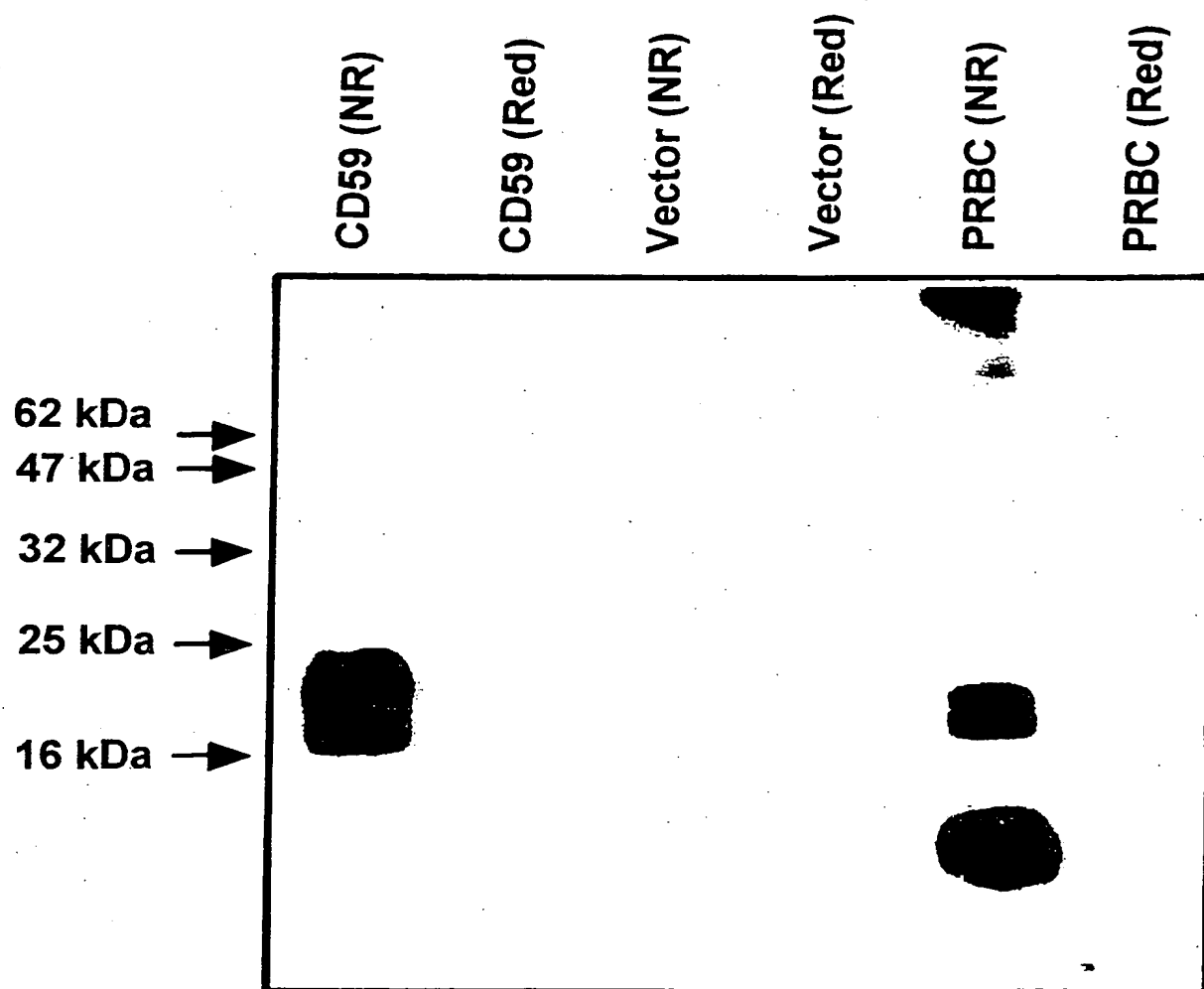
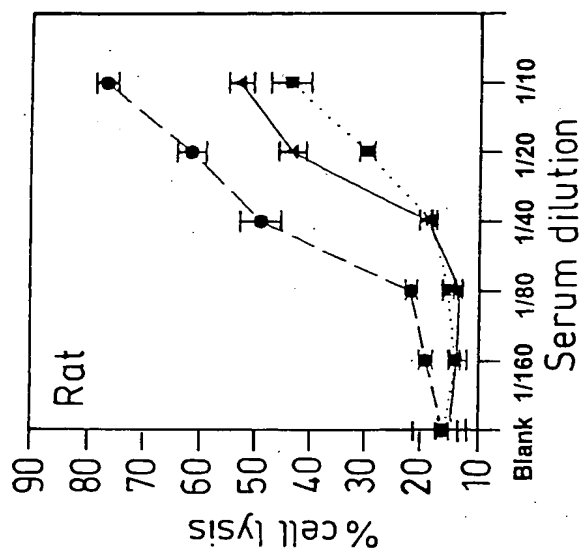
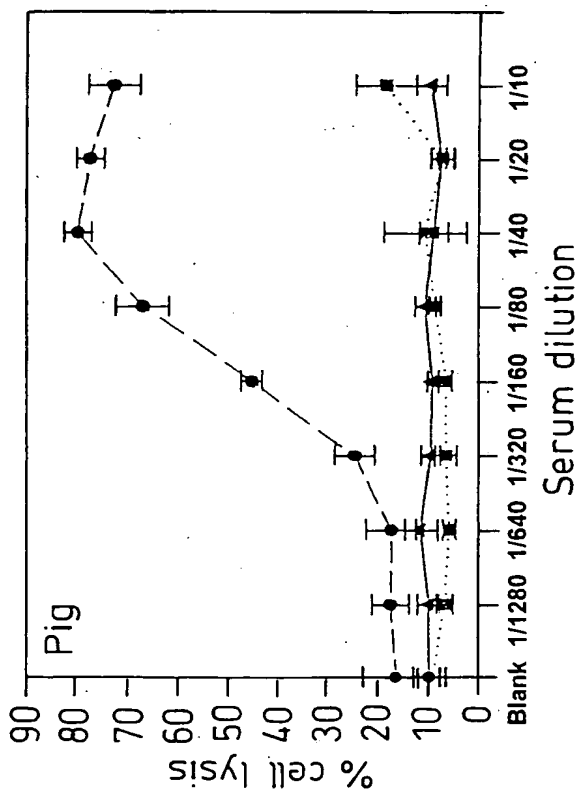
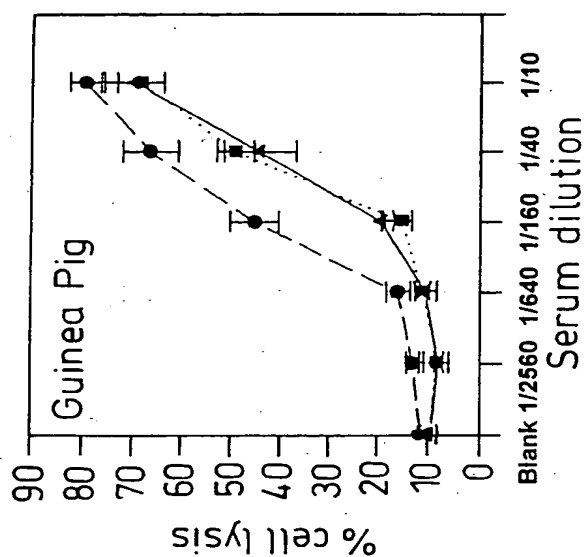
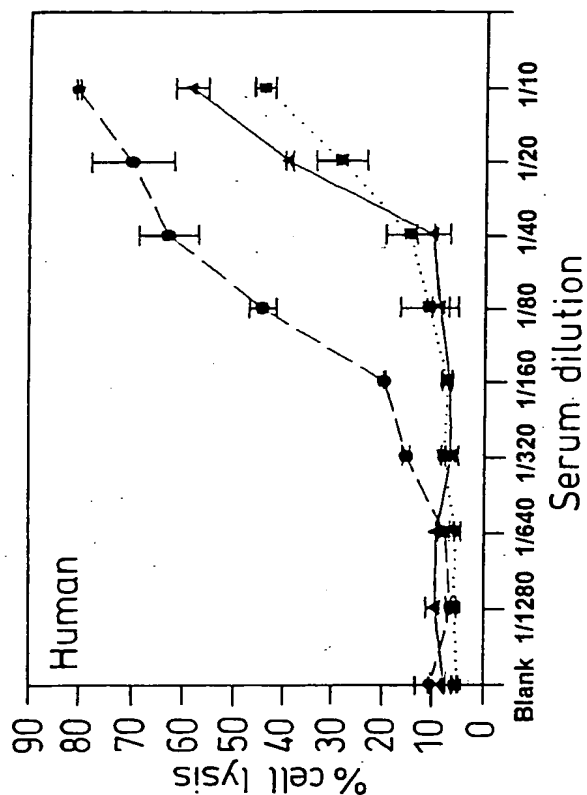


Fig. 6

Fig. 7 (part 1 of 2)

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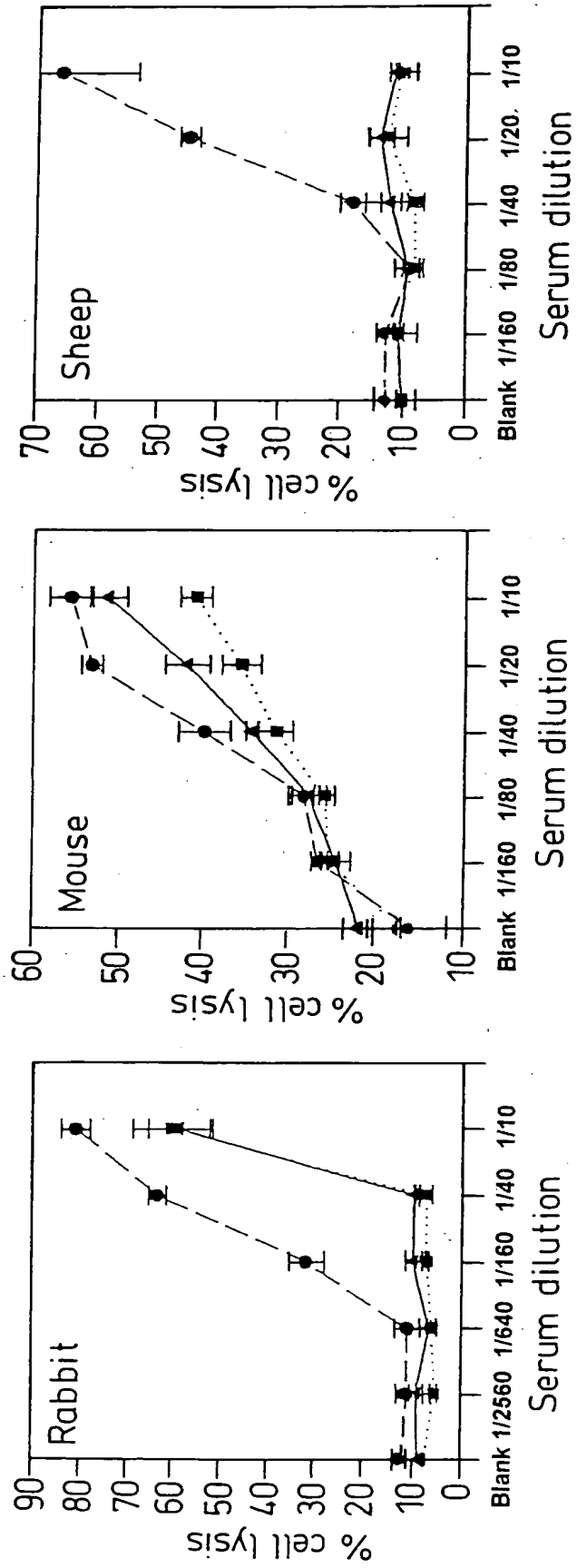
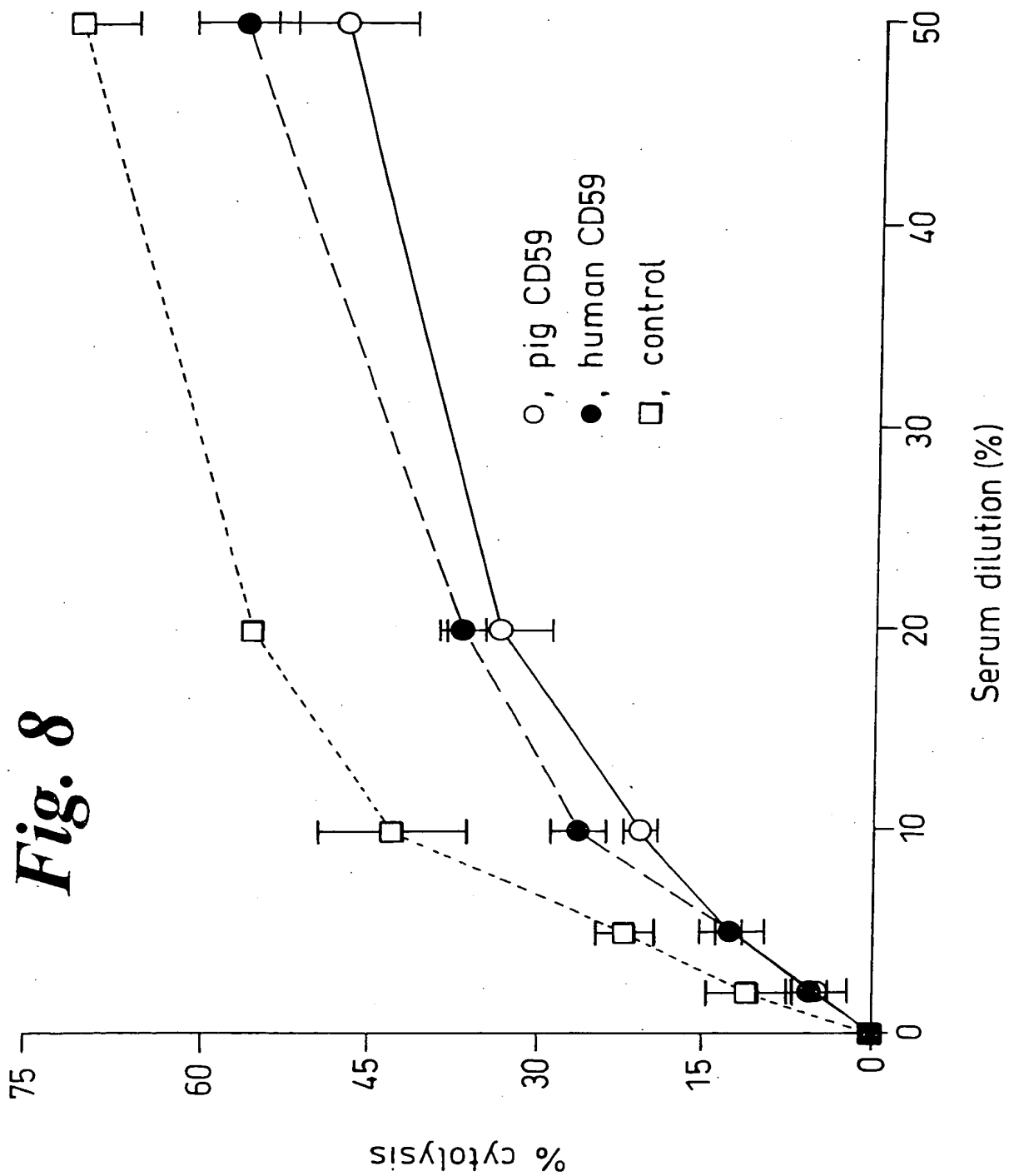


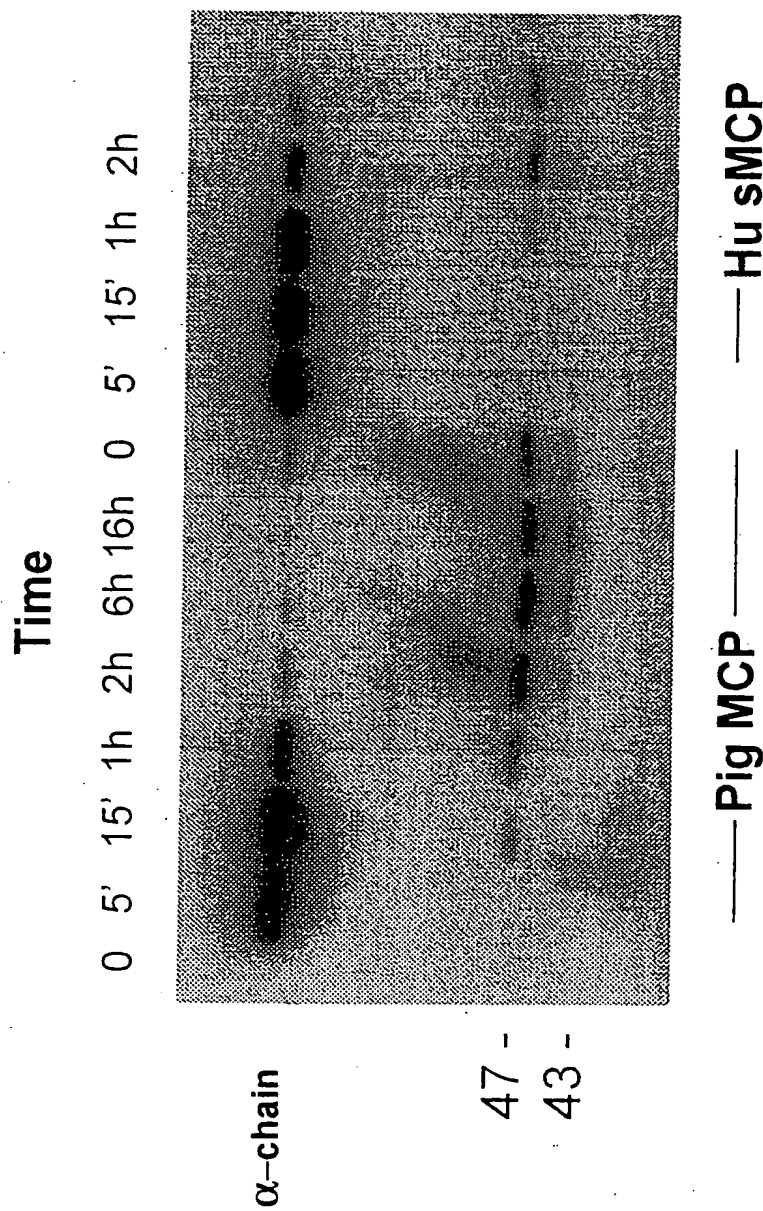
Fig. 7 (part 2 of 2)

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Time course Cofactor activity: pig MCP vs Hu sMCP

Fig. 9



500 ng C3 was incubated with 50 ng factor I and 50 ng pig MCP or human sMCP

Pig MCP is a better cofactor than Hu sMCP for human C3 and human factor I

Dose/response Cofactor activity: pig MCP vs Hu sMCP

300 100 30 10 3 1 - 300 100 30 10 3 1 - ng MCP



Fig. 10

— Pig MCP — Hu sMCP —

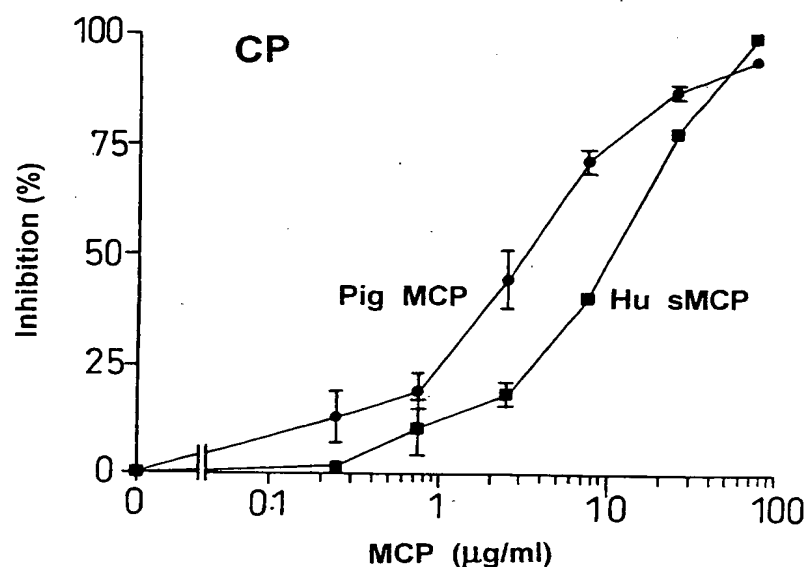
500 ng C3 was incubated with 50 ng factor I and various amounts of pig MCP or human sMCP for 16 at 37°C. W.blot of reduced samples, probed with anti Hu C3c

Pig MCP is a better cofactor than Hu sMCP for human C3 and human factor I

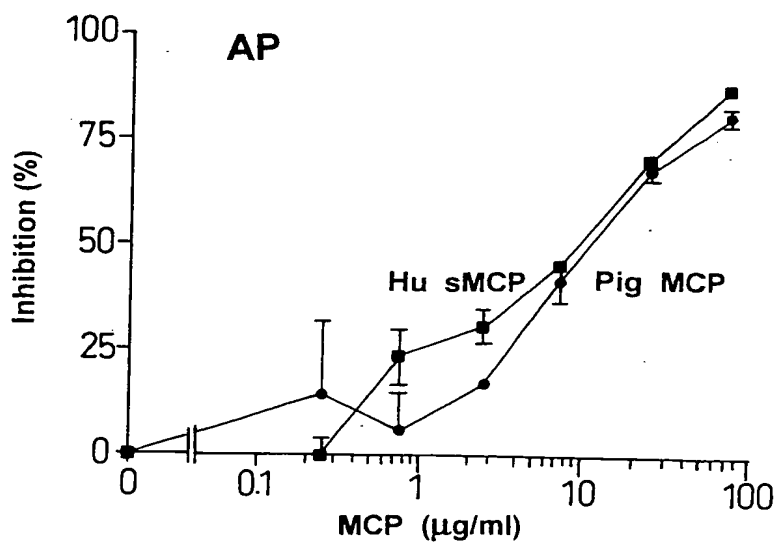
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Fig. 11

**Inhibition of CP and AP of human serum
by human sMCP and pig MCP**



RaE were incubated with human serum in the presence of Hu soluble MCP or pig MCP under CP or AP conditions.



Pig MCP is a better regulator of the CP of human C than human sMCP.

Pig MCP and Hu sMCP have similar activity in regulation of the human AP.

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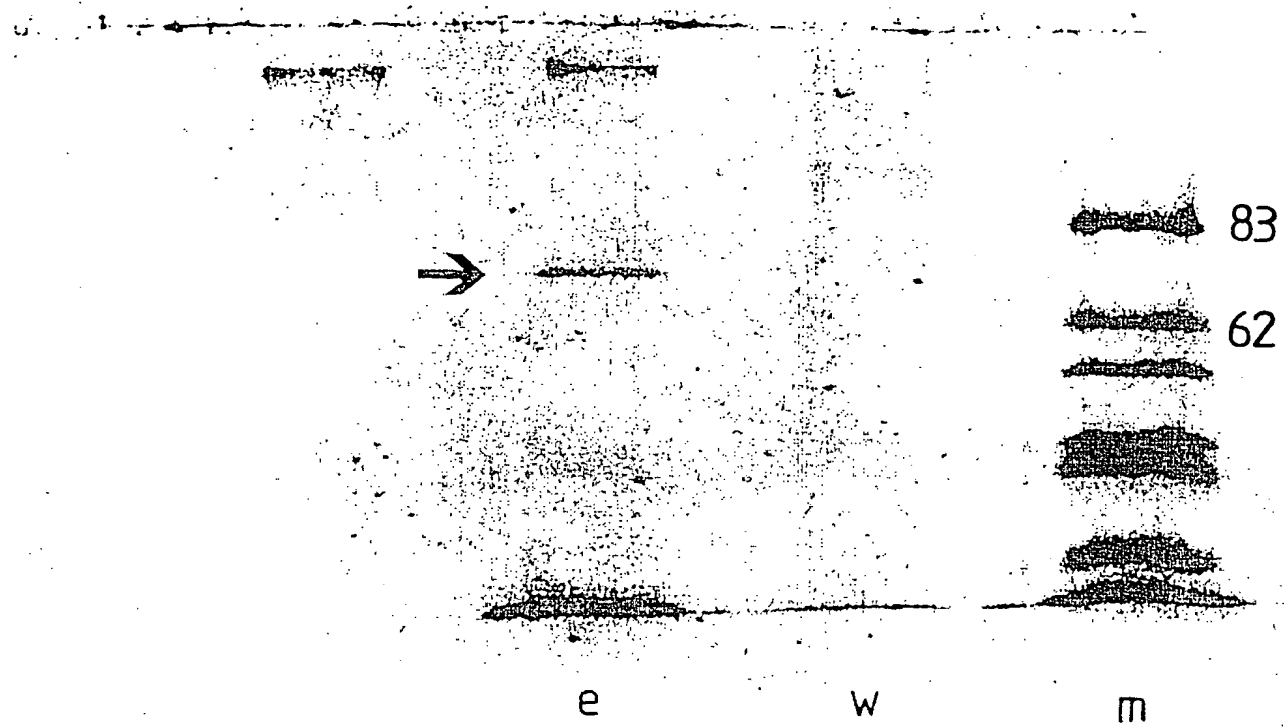


Fig. 12

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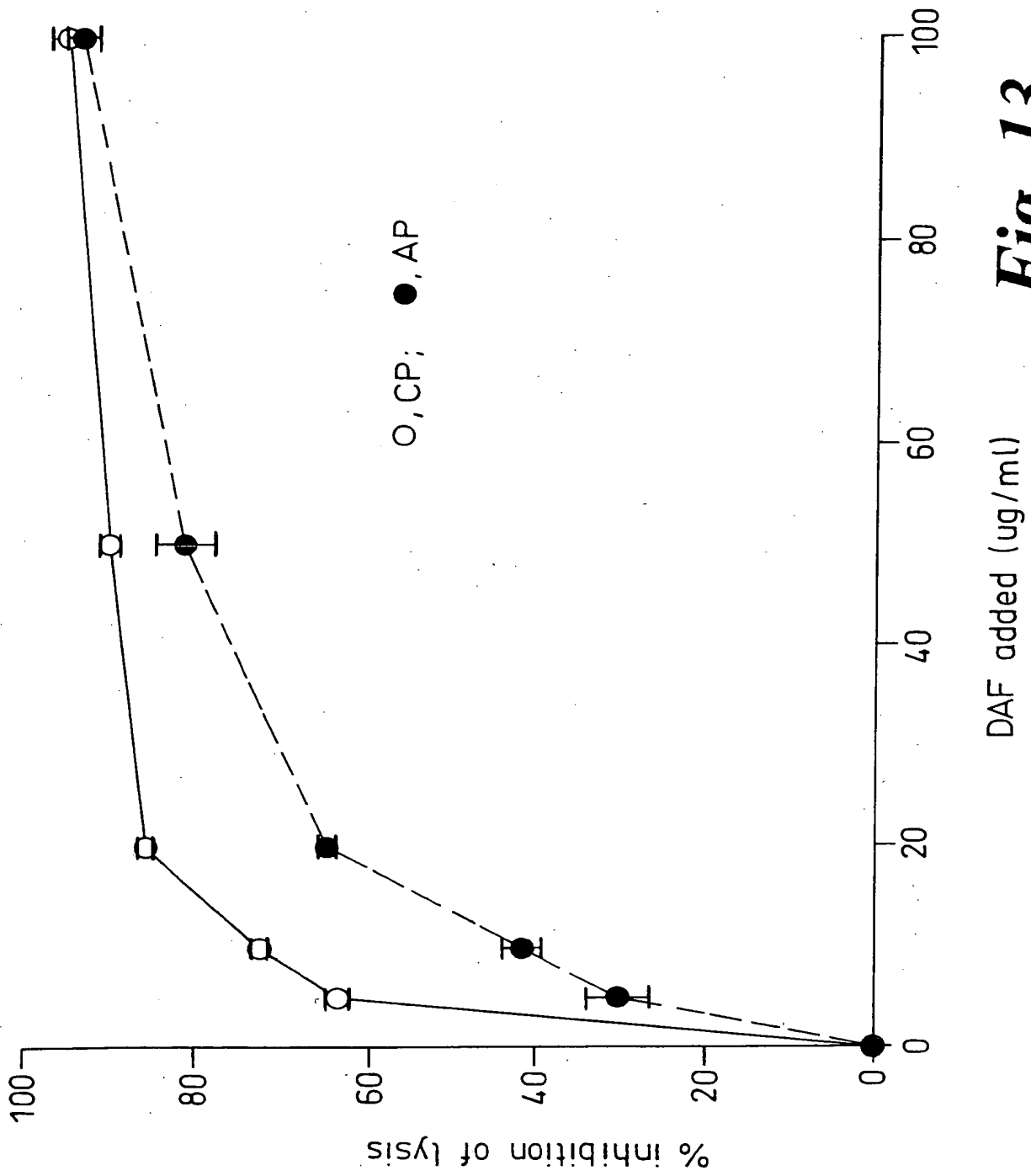


Fig. 13

pDAF-7 cDNA sequence:

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 GCCGCTCGGGGTCCCCGTTCCCGCGCGCCATGAGTCCCCGCGCGGAGC
 GCGCCCGCGGGTGAGGCGCCTAATGGGCGGACAGACGCCGCCGCCGCTGCT
 GCTGCTGCTGCTGCTGCTGCTGTGTATCCCGGCTGCGCAGGGTGACTGCAGCC
 TTCCACCCGATGTACCTAATGCCCAACCAGATTTGCGAGGTCTTGCAAGT
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 GCCATAGCAAATGATGTTGAGTGGAGTGATCCATTGCCAGATTGCCAAGA
 AATTTCTCCAACGTCAAAGCCATACCAGCTGTTGAGAAACCCATCACAG
 TAAATTTTCCAGCAACAAAGTATCCAGCTATTCCCAGGGCCACAACGAGT
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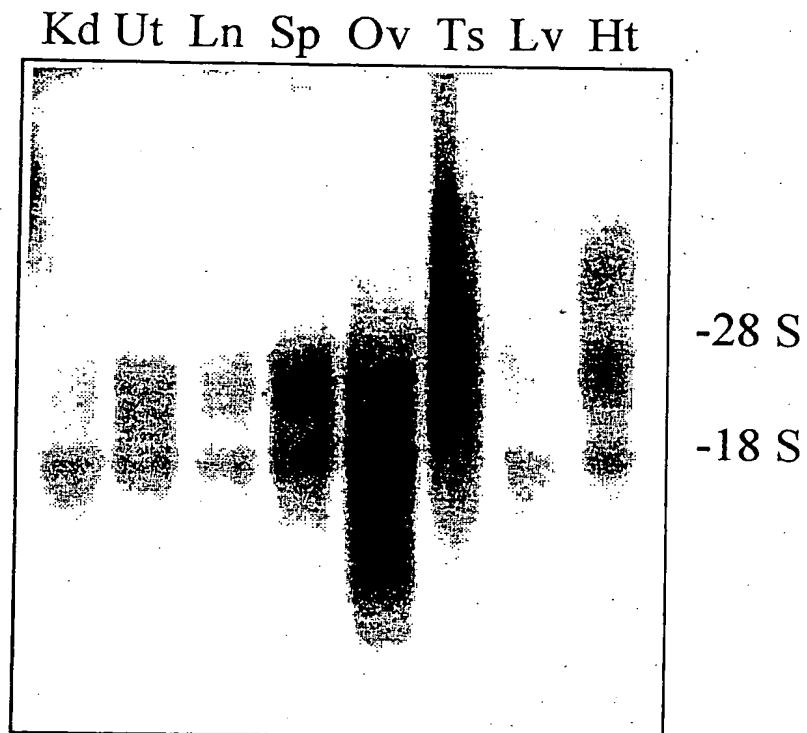
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 TCTTAATGATAAATGGTCAGAAGTTGCAGAATTTTGTAAATCGTAGCTGTG
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 CAGAATTATTTCCAGAGGGTTTACCCTGGAATATGAGTGCCGTAAGGG
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 TTACGTGGTCCAAACCTGATGAATTTTGCAAAAAAACAATGTCCGACT
 CCTGGAGAATAAAAAATGGTCATGTCAATATAACAACCTGACTTGTATT
 TGGCGCATCCATCTTTTTCTCATGTAACGCAGGGTACAGACTAGTTGGTG
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 CCATTGCCAGAAATGCCAAGAAATTTCTCCAACGTCAAAGCCATACCAGC
 TGTTGAGAAACCCATCACAGTAAATTTCCAGGTACCAAAGCCCTATCAT
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 CAACTCCTCAGGAACCCACCACAGTAAATGTTCCAGATAGTAAAGCCATA
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 CCAACTCCTCAGGAACCCACCACAGTAA

Fig 14

Fig 15

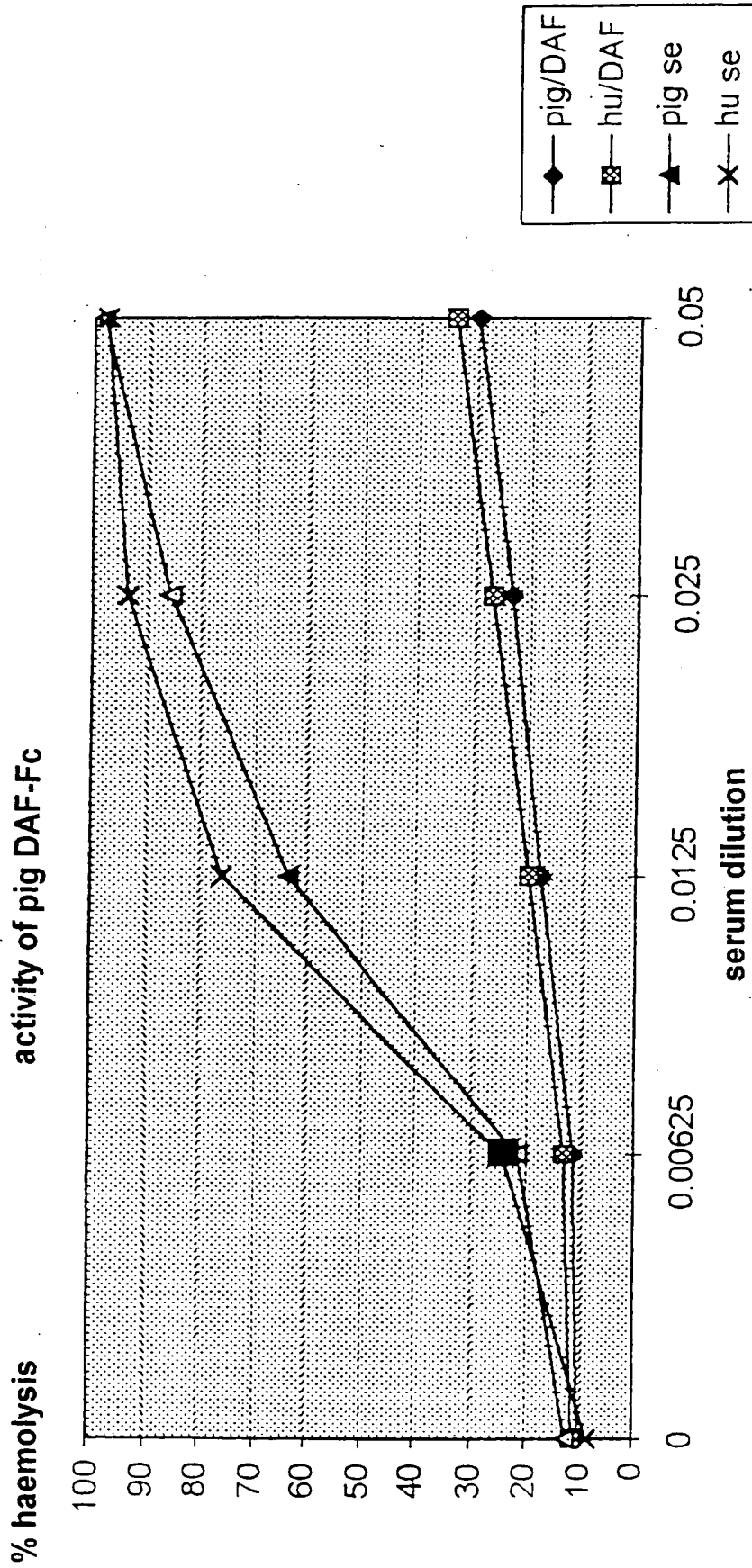
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Northern analysis of porcine DAF

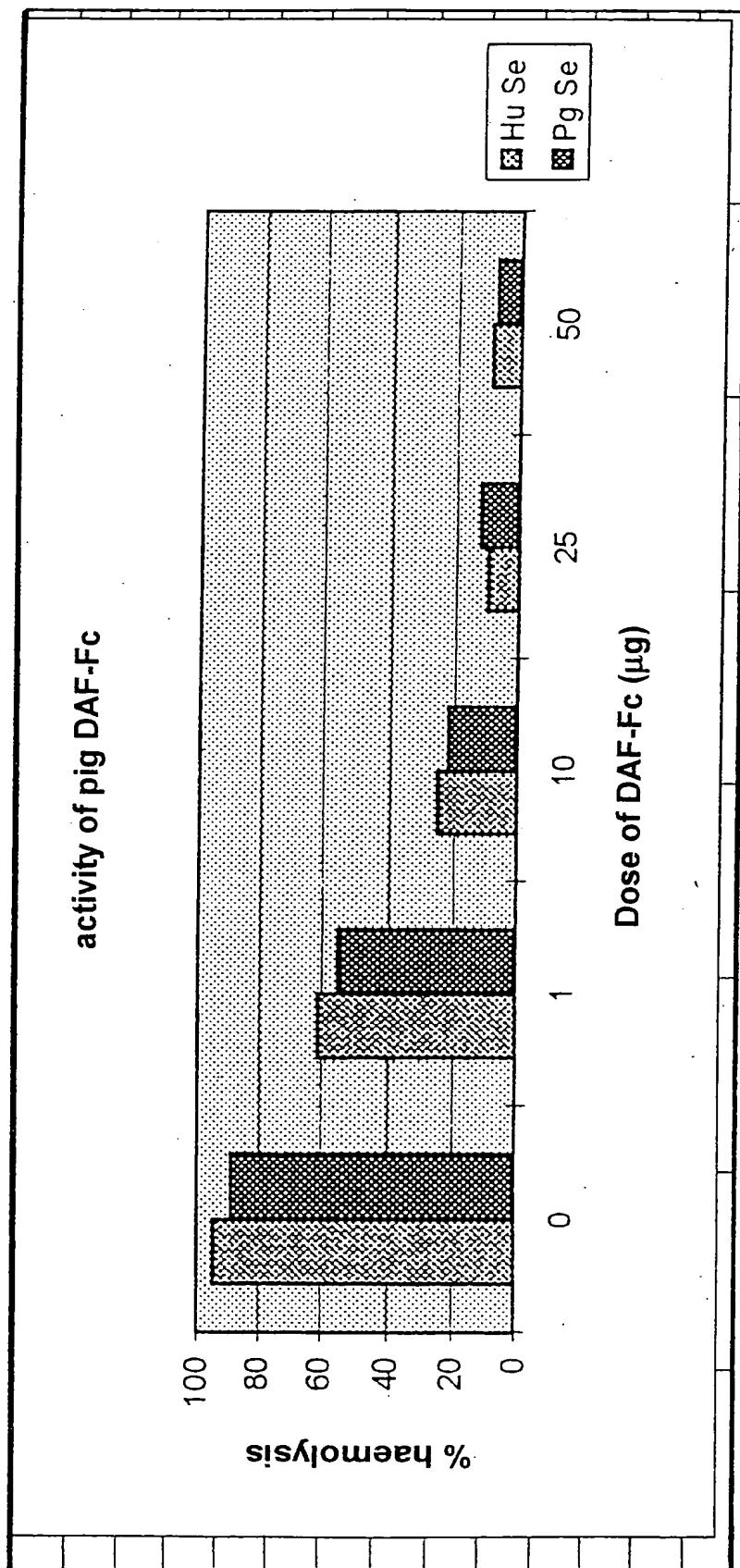
Fig. 16

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Antibody-sensitised human erythrocytes in GVB were incubated for 30 min at 37° C with various dilutions of pig or human serum in the presence or absence of pig DAF-Fc at 10µg/ml (final). Haemolysis was measured by quantifying haemoglobin release into supernatant.

Fig 17a Activity of pig DAF-Fc



Antibody-sensitised human erythrocytes in GVB were incubated for 30 min at 37° C with a constant dilution of human or pig serum (1:20) and various amounts of pig DAF-Fc (0 - 50µg/ml (final)). Haemolysis was measured by quantifying haemoglobin release into supernatant.

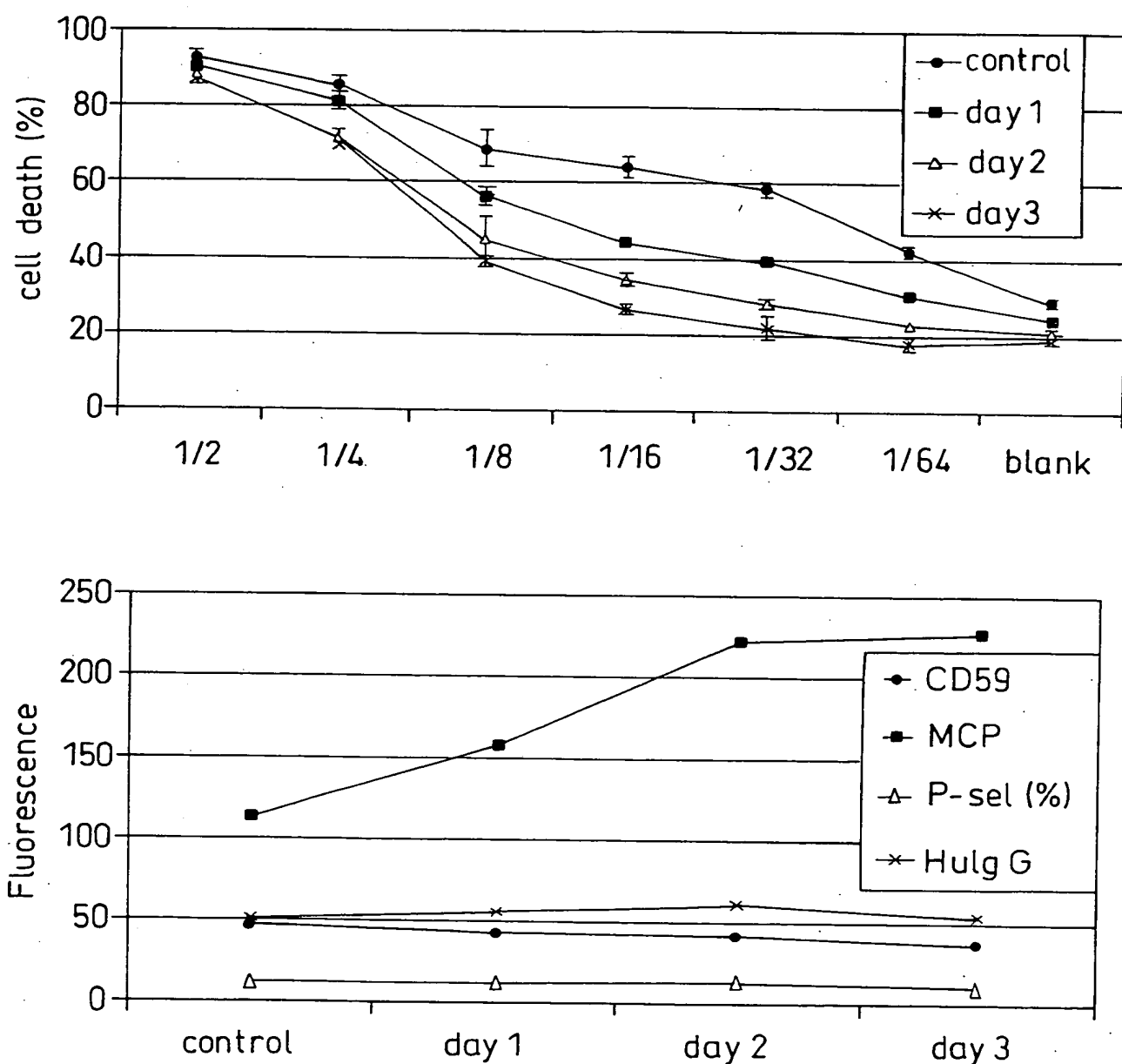
Fig. 17b Activity of pig DAF-Fc - dose response with human and pig serum

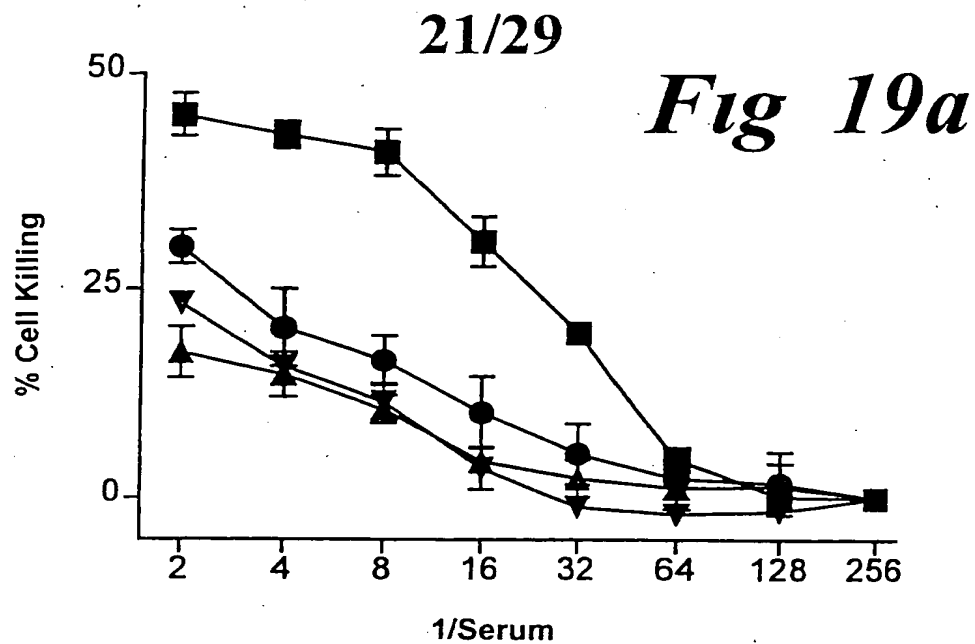
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Effect of PMA on expression of CD59 and MCP and C- susceptibility of PAEC

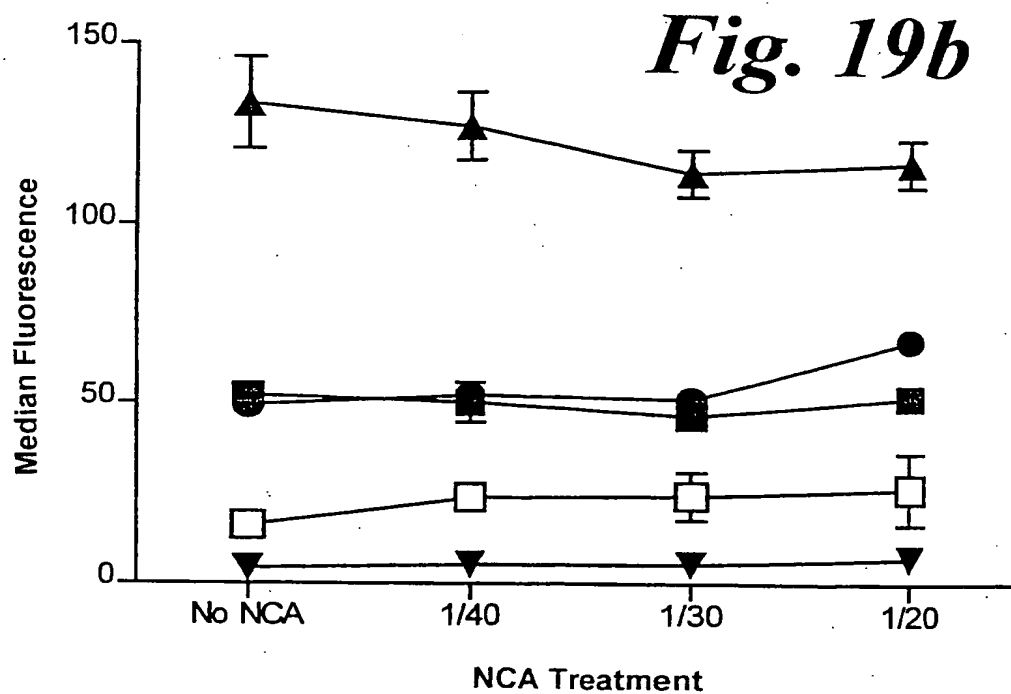
Fig 18

PAEC were cultured in the presence of 10 nM PMA. Cells were harvested and analysed for expression of pig CD59 and pig MCP and other cell surface markers and susceptibility to lysis by NHS



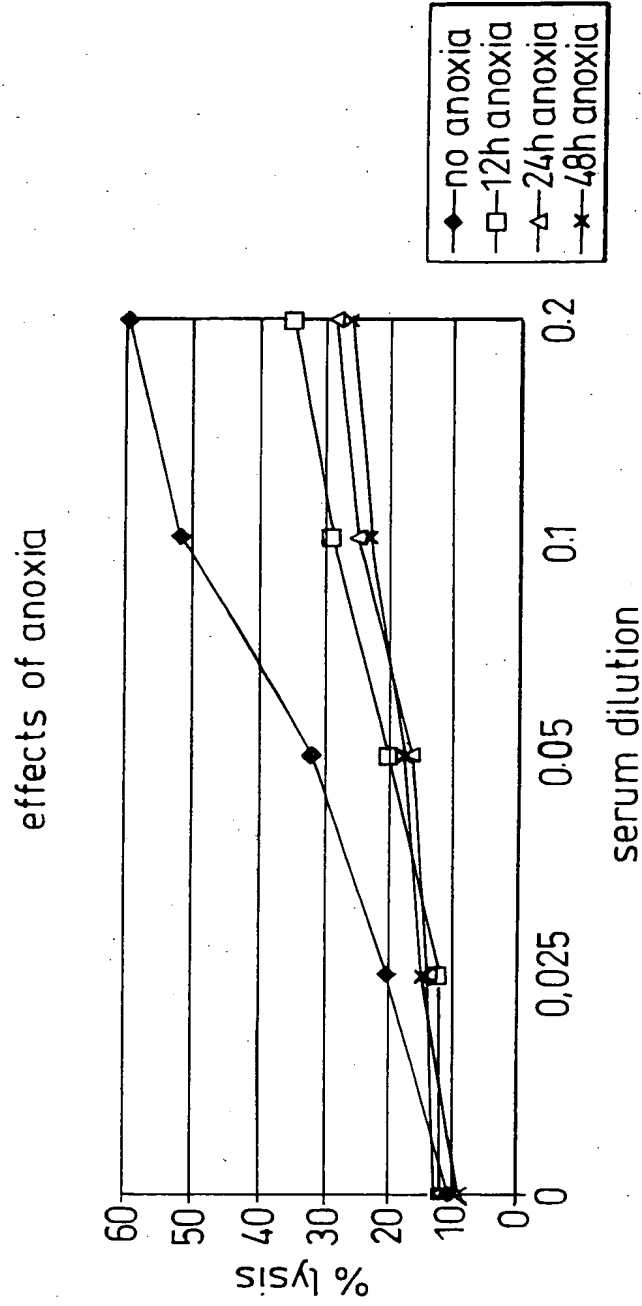


Effect Of Non-Lethal Complement Attack on the Lysis Of PAE cells PAE cells were incubated with 1/20 (▲), 1/30 (▼), 1/40 (●) or zero human serum (■) before being used in a propidium iodide cell killing assay against NHS. Values are means of triplicates \pm SD.



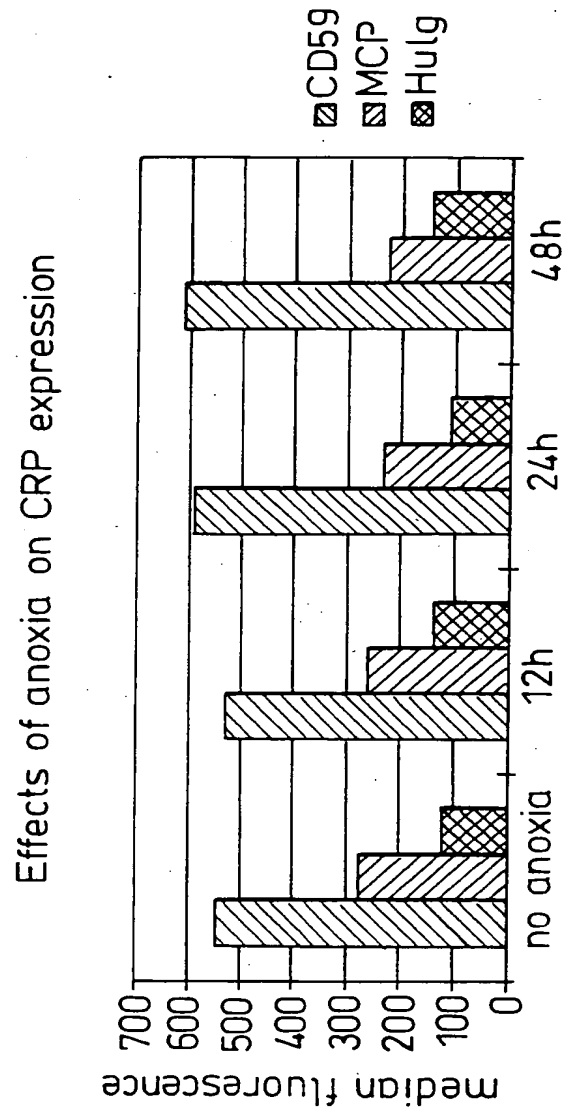
Staining of NCA Treated PAE Cells Sensitised PAE cells were incubated with different non-lethal concentrations of human serum. These cells were then stained for MCP (■), Human IgG (□), CD59 (▲), P-selectin (total cells) (□) or P-selectin (positive staining cells) (▼). Values are means of triplicates \pm SD.

Fig. 20a Effects of anoxia



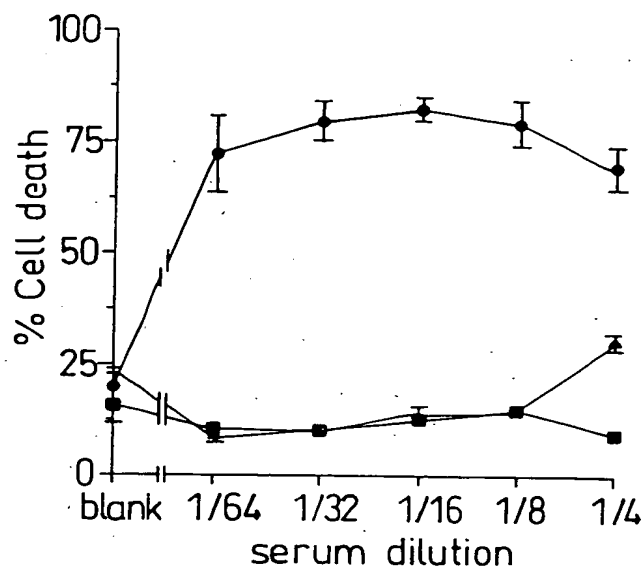
PAEC were incubated under anoxic conditions at 37° C for 0, 12 or 48 hours. Cells were then subjected to complement attack by exposing to various dilutions of human serum

Fig. 20b Effects of anoxia



PAEC were incubated under anoxic conditions at 37° C for 0, 12 24 or 48 hours. Cells were then analysed by flow cytometry for expression of CD59, MCP or binding of Hulg.

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a: K562 cells were growth-arrested either by nutrient deprivation (triangles) or by maintaining at confluence in culture (squares). Control cells (circles) had been maintained in log growth in normal medium. Cells were then antibody sensitised and exposed to various dilutions of human serum. End-point lysis was measured at 60 min.

b: Cells growth arrest as above were stained for the various complement inhibitors and analysed on the FACScan. Open bar; control; hatched bar; confluence; solid bar; nutrient deprived. All points are mean \pm SD of triplicates.

Fig. 21a

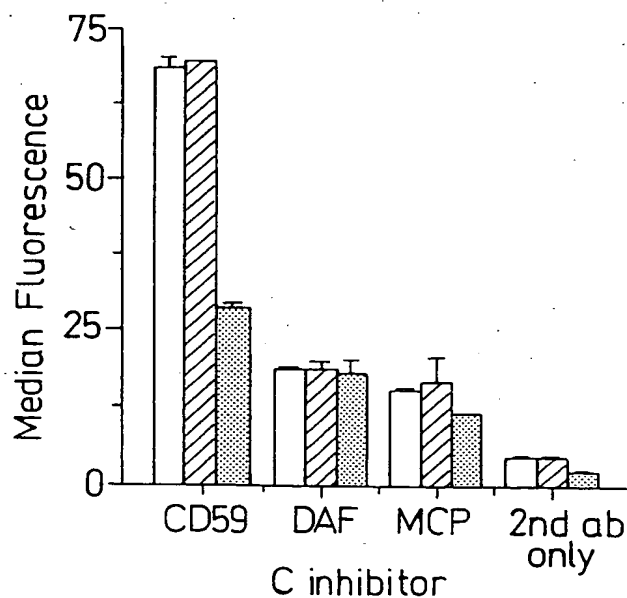


Fig 21b

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**Expression of pig CD59 on pig
aortic endothelial cells (PAEC) at
different passages.**

Cells were harvested from pig aortae and cultured. Cells were stained for pig CD59 using mAb's Mel2 and Mel3. after 1 day culturing (Primary) or after subculturing (P1-P5, appr. 4-7 days between passages).

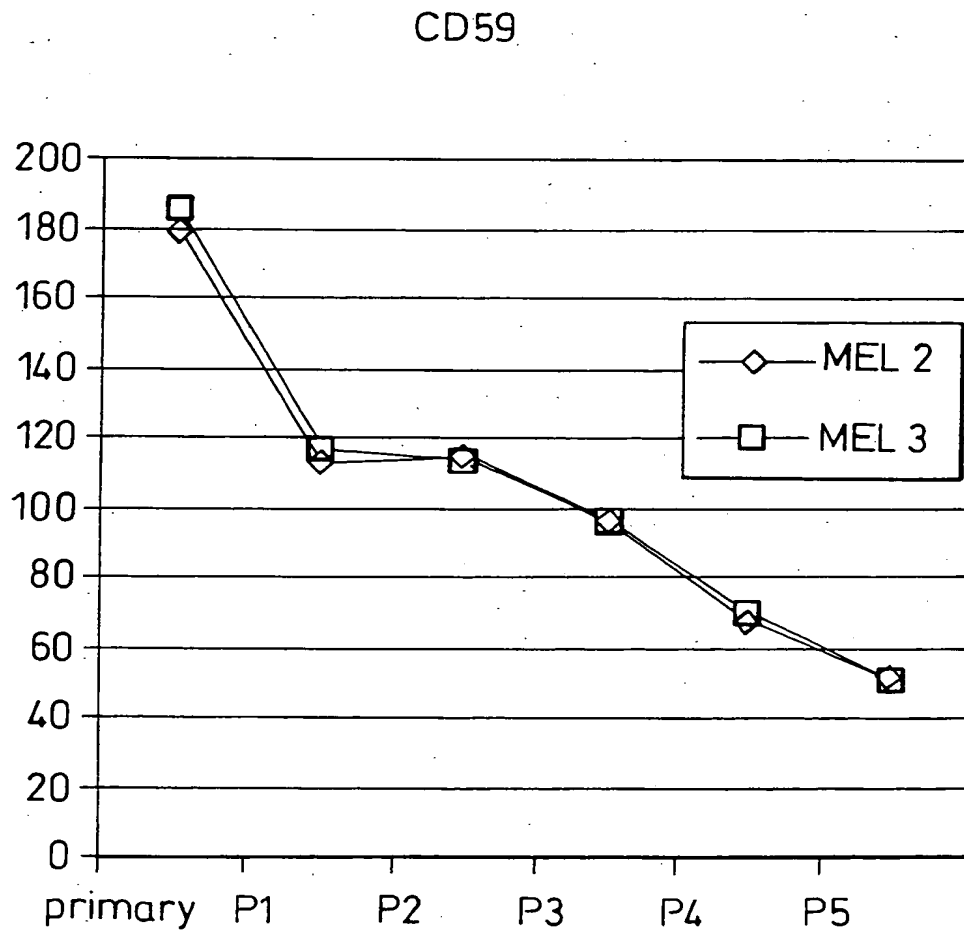


Fig 22

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**Expression of pig MCP on pig
aortic endothelial cells (PAEC) at
different passages.**

Cells were harvested from pig aortae and cultured. Cells were stained for pig CD59 using mAb's 4C8 and 1C5. after 1 day culturing (Primary) or after subculturing (P1-P5, appr. 7 days between passages).

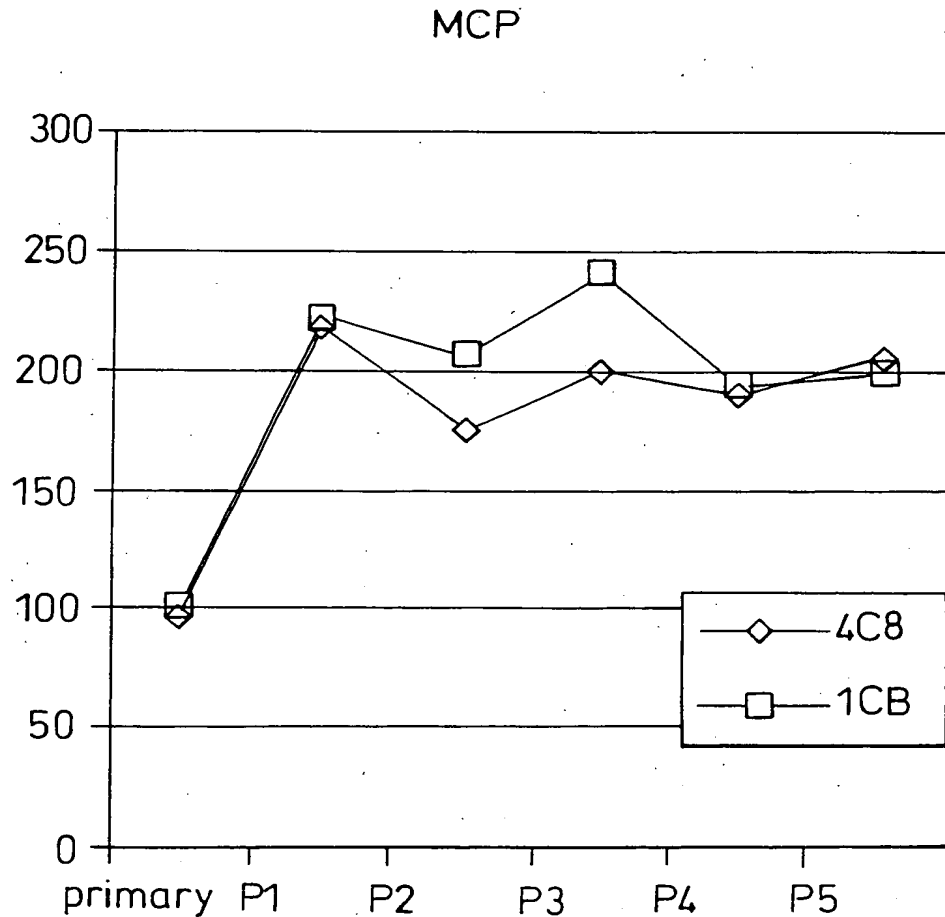


Fig 23

C-susceptibility of pig aortic endothelial cells (PAEC) at different passages.

Cells were harvested from pig aortae and cultured. Cells assayed for C-susceptibility after 1 day culturing (Primary) or after subculturing (P2 and P5). The cells were also analysed for the expression of CD59, MCP and binding of human Ig

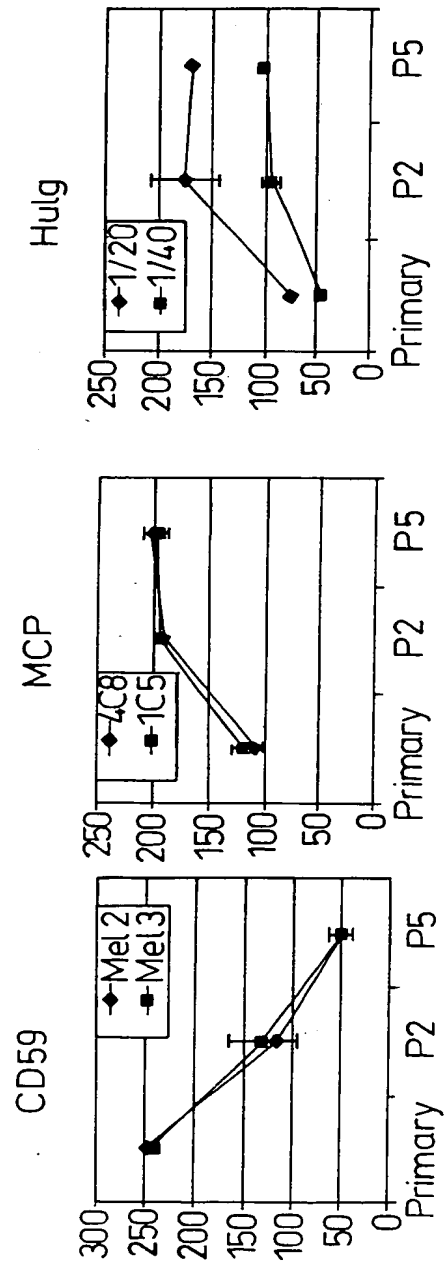
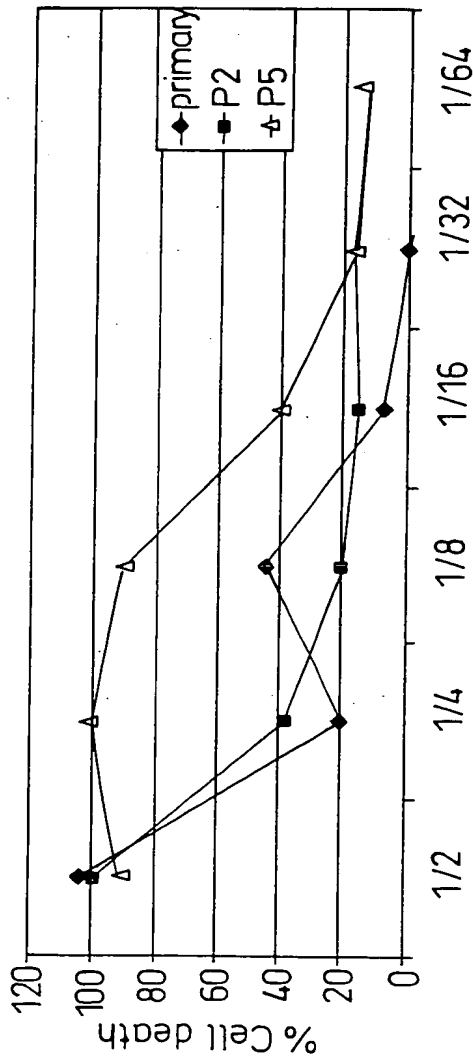


Fig. 24

Effect of blocking CD59 and MCP of C-susceptibility of PAEC.

PAEC were incubated with blocking Ab's against CD59 and MCP and C-susceptibility was assessed after challenging with HuS

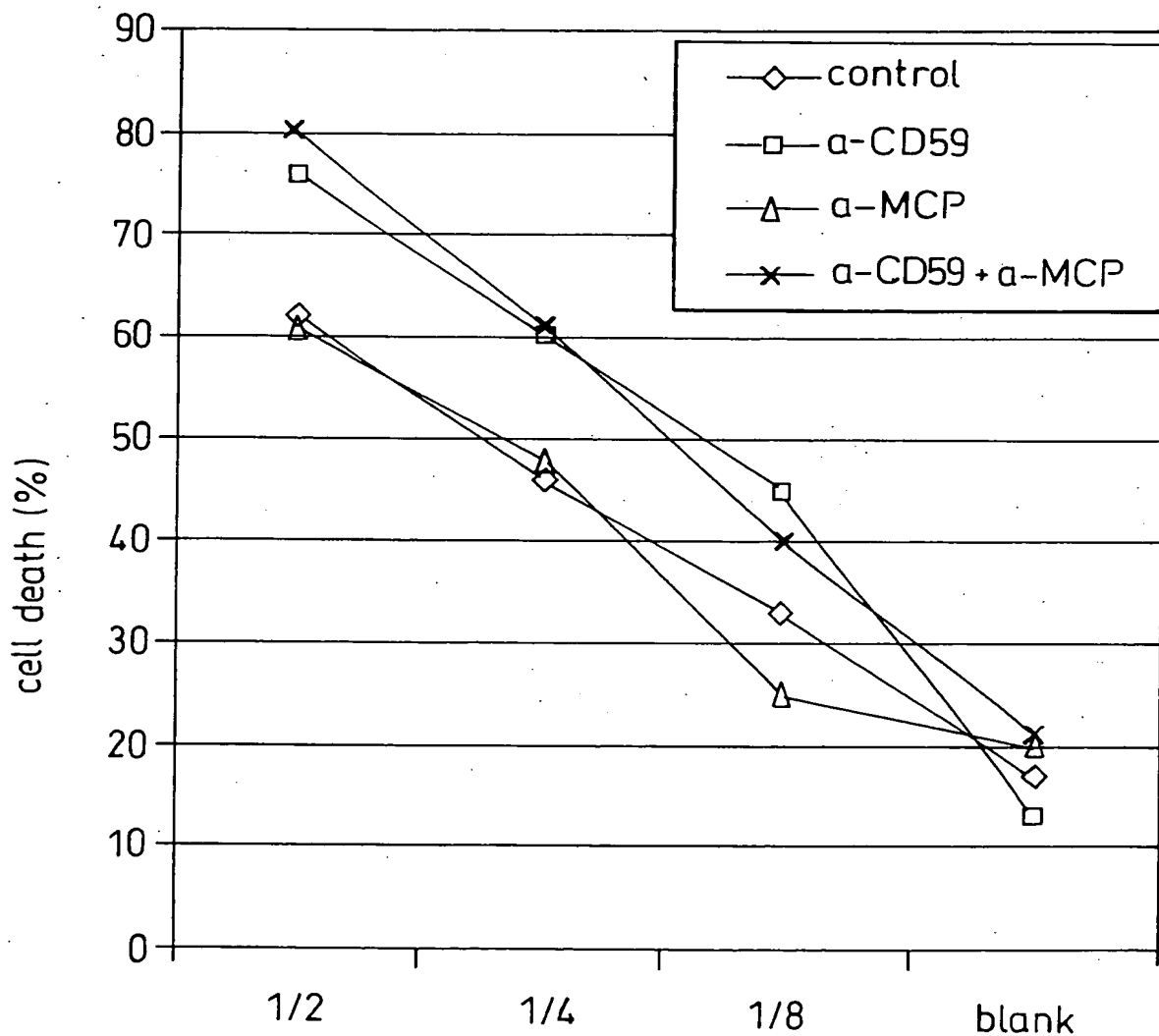


Fig 25

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Incorporation of Human CD59 into PAEC and effect of blocking of human and pig CD59 on C-susceptibility.

PAEC were incubated with 1 $\mu\text{g/ml}$ CD59 for 30 min and followed by incubation with blocking antibodies against Human CD59 (Bric229) and pig CD59 (Mel2). Cells were assayed for C-susceptibility and levels of pig and human CD59

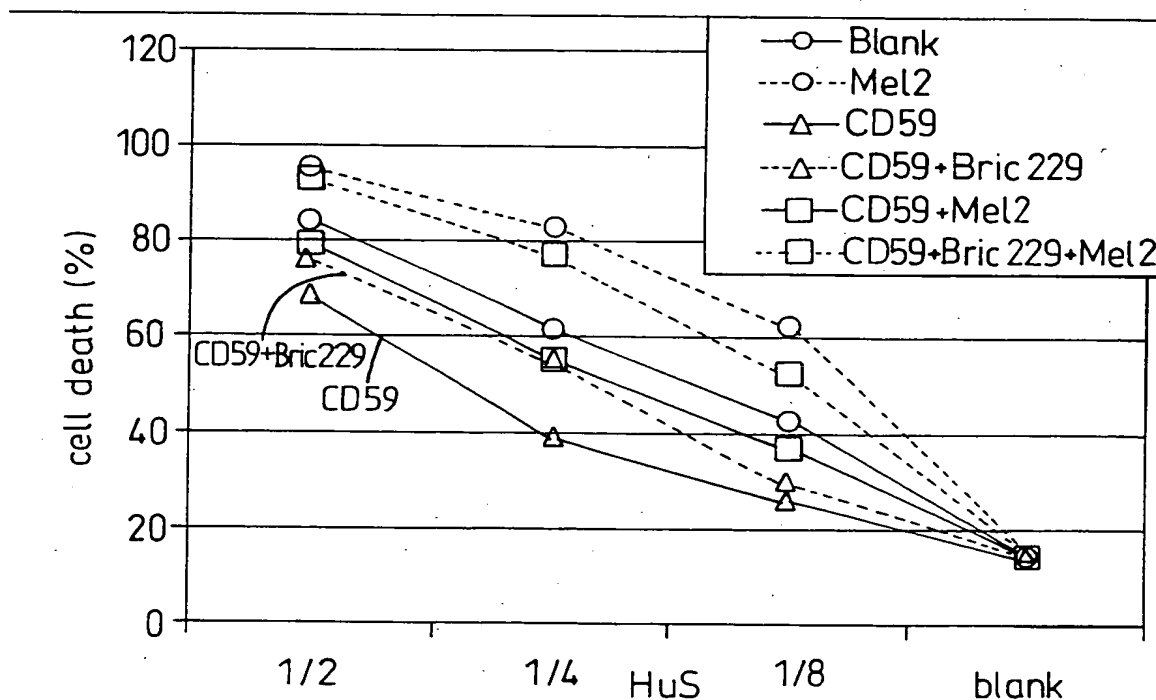
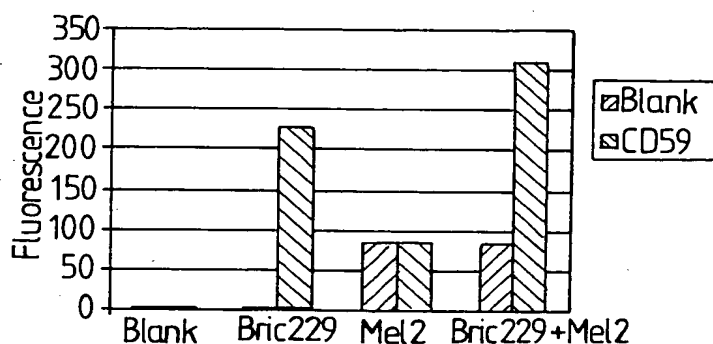


Fig. 26